replacement of water donor/acceptor H-bonds. The lack of unstable solvation is consistent with the observed rapid exit of OmpC substrates. We docked the six antibiotics into the crystal structure of porin OmpC, and compared the overlay between those molecules and the calculated solvation map. The results suggest that rapid OmpC translocation is limited to substrates with exposed polar groups capable of replacing several simultaneous water-protein and water-water H-bonds. This is consistent with the known physico-chemical properties of the majority of Gram-negative antibiotics, and in particular, the hydrophilic nature of these molecules.

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Activity of AmB along a Phase Diagram Presenting Micro and Nanodomains

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Polyene antimicotics have been the most effective drug for more than five decades, and at the same time their mechanism of action is one of the most discussed biological processes. Understanding the molecular mode of action is essential not only for advancing our knowledge of transmembrane transport but also in the search for new derivatives with lower toxicity. Recently, two articles have stated that the mechanism of action of Amphotericine B has been resolved, proposing that the direct interaction of AmB with the sterols is responsible for selectivity towards fungal membranes (PNAS 1015023108, JACS 132,18266,2010). In our group, we have been advancing the idea that membrane structure is responsible for the drug selectivity, and two of our recent articles have demonstrated a strong correlation between the phase diagram of the lipidic membrane and the action of Nystatine (J. Memb. Biol. 2010,237,41-49 and 31-40). In order to continue the search for understanding the molecular mechanism of action, we performed an study of the activity of Amphotericine along a the phase diagram of ternary and quaternary mixtures of DSPC/DOPC/POPC/Chol that have shown to produce nano and microdomains in different parts of the phase diagram (Konyakhina et al., Biophy J, 101, L08-L10, 2011). We found again a strong correlation between membrane structure and polyene activity. Given the presence of different membrane domains, different levels of activity have been found in the formation of ionic channels with the patch-clamp technique.

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Using Lithium to Probe Sequential Cation Interactions with GAT-1

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Li^+ interacts with the Na^+/Cl^--dependent GABA transporter, GAT-1 under two conditions: in the absence of Na^+ it induces a voltage gated leak current; and in the presence of Na^+ and GABA, Li^+ increases the apparent Na^+ affinity (MacAulay *et al*. J Physiol. 2002, Kanner JBC, 2003). The amino acids directly involved in the interaction with the Na^+ and Li^+ ions in the so called ''*Na2* binding site'' have been identified (Zhou *et al*. JBC 2006), but how Li^+ affects the kinetics of GABA cotransporthas not yet been explored. We expressed GAT-1 in *Xenopus* oocytes and applied two-electrode voltage clamp and ^{22}Na uptake assays to determine coupling ratios and steady-state and presteady-state kinetics under experimental conditions in which extracellular Na^+ was partially substituted by Li^+. The three major findings were: *i*) Li^+ reduced the coupling ratio between Na^+ and ''net charge'' translocated during GABA cotransport, *ii*) Li^+ increased the apparent Na^+ affinity without changing its voltage dependence, *iii*) Li^+ altered the voltage dependence and time course of cation-interactions in the absence of GABA. These findings have allowed us to dissect the kinetics of the two Na^+ binding sites. We propose an ordered binding scheme for cotransport in which either a Na^+ or Li^+ ion can bind at the putative first cation binding site (Na2). This is followed by the cooperative binding of the second Na^+ ion (Na1 binding site) and then GABA. With Li^+ bound in the first ''low affinity'' binding site, the second Na^+ ion is more readily bound to the protein, and despite a lower GABA affinity, the translocation rate of the fully loaded carrier is not reduced. Model simulations confirmed that a sequential cation binding scheme was most appropriate to fully describe the experimental data.

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Insertion of Colicin E1 into Omp TolC is Mediated by the Ordered Segment of Translocation Domain

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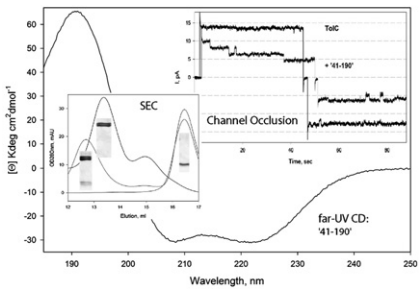
Cellular import of nuclease colicins through the outer membrane utilizes the OmpF porin; crystal structures imply that a disordered N-terminal segment initiates insertion[1,2]. Colicin E1 utilizes the drug export protein, TolC. Recombinant colicin peptides spanning the N-terminal translocation and receptor-binding domains were used to define TolC-binding site(s). Far-UV CD spectra showed that the N-terminal 40 residue segment lacks ordered secondary structure, while near-UV spectra imply tight packing around aromatic residues. Peptide '41-190' is helical (78%). High helix content and significant cooperativity of melting of receptor(BtuB)-binding domain implies a coiled-coil conformation. Binding of colicin N-terminal peptides to TolC was tested using size-exclusion chromatography and occlusion of TolC channels[3]. The '41-190' peptide co-eluted with TolC, whereas '1-81' peptide and TolC eluted separately. Channel activity was assayed with TolC reconstituted into planar bilayers. While the '41-190' peptide caused closure of TolC channels, the '1-40' and '1-81' peptides did not occlude. Peptides '82-140' and '141-190' were active in TolC channel closure, although less efficiently than peptide '41-190' [NIH GM18457; Henry Koffler Professorship].



[1]EMBO J., 27, 2171-2180, 2008;
[2]PNAS, 107, 21412-21417, 2010;
[3]Biophys. J., 87, 3901-3911, 2004.

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Molecular Determinants for Subtype-Selective Ion Channel Block of NMDA Receptors by Argiotoxin Analogs

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The NMDA-type of ionotropic glutamate receptors (iGluRs) are involved in excitatory transmission in the mammalian brain and in a range of neurological and psychiatric diseases for which NMDA receptors are considered potential therapeutic drug targets. The majority of NMDA receptor subtypes are comprised of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits (NR2A-D); leading to the existence of four major receptor subtypes with distinct expression patterns and functional properties: GluN1/2A, GluN1/2B, GluN1/2C, and GluN1/2D. While the GluN1/2B subtype can be selectively inhibited by the highly subtype-selective non-competitive antagonist ifenprodile, the lack of inhibitors with similar subtype selectivity for the GluN1/2A, GluN1/2C, and GluN1/2D receptors is a limitation in studies exploring the role of these subtypes in many aspects of brain function and disease.

Polyamine toxins isolated from the venom of spiders are open-channel blockers of ion channels, in particular iGluRs. Argiotoxin-636 (ArgTX-636) isolated from the venom of the orb weaver spider Argiope lobata consists of an aromatic amino acid head-group coupled to a polyamine tail. ArgTX-636 is a potent inhibitor of mammalian iGluRs; presumably by binding to the ion channel region in a use- and voltage-dependent manner that prevents ion conduction. We have recently shown1 that modifications within the polyamine tail of ArgTX-636 can control selectivity between the NMDA- and the AMPA-type of iGluRs (1). Further exploration of the analog lead-structures have recently developed a series of analogs of the spider toxin Argiotoxin-636 (ArgTX-636), which display robust selectivity towards GluN1/2A and GluN1/2B over GluN1/2C, and GluN1/2D subtypes of NMDA receptors. In this study we have explorer the molecular determinants for subtype-selectivity of the novel analogs ArgTX-48 and ArgTX-75 within the NMDA receptor ion channel using electrophysiological characterization of chimeric and mutant GluN1/2A and GluN1/2D NMDA receptors.